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Prospective study of verocytotoxin-producing, enteroaggregative and diffusely adherent *Escherichia coli* in different diarrhoeal states

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SUMMARY

One hundred and eighty-one stool specimens from patients with various types of diarrhoea (135 patients) or from non-diarrhoeal controls (23 acute medical patients, 23 inflammatory bowel disease in remission) were investigated using a colony-blot DNA hybridization assay for the presence of Verocytotoxin-producing (VTEC), enteroaggregative (EAggEC) and diffusely adherent (DAEC) *Escherichia coli*. Twelve patients had probe-positive EAggEC in the stool and 8 of these had diarrhoea, 6 following recent travel. Eight patients had DAEC, 7 of whom had travellers' diarrhoea. Six of 10 (60%) travellers with gastroenteritis, but without a recognized enteric pathogen, were positive for EAggEC (4) or DAEC (2). Five of 10 (50%) travellers with gastroenteritis related to a recognized enteric pathogen also had DAEC identified in their stool. Of the 23 acute medical control patients 11 had been abroad, 4 of these were immigrants and had EAggEC. VTEC were not found and, with one exception, immunoassays for antibodies to *E. coli* O 157 and O 2 lipopolysaccharides were negative.

INTRODUCTION

There are at present four recognized subgroups of diarrhoea-causing *Escherichia coli*, namely: enteropathogenic, enterotoxigenic, enteroinvasive and Verocytotoxin-producing (enterohaemorrhagic) types. There is evidence that *E. coli* showing aggregative adhesion (EAggEC) or diffuse adhesion (DAEC) are also bowel pathogens [1–4]. EAggEC and DAEC have been associated with childhood gastroenteritis in the tropics and in the case of EAggEC the diarrhoea is often haemorrhagic and prolonged [3–5]. VTEC are recognized as a common food poisoning pathogen in North America [6] and the major cause of haemolytic–uraemic syndrome in most developed countries [7, 8]. VTEC, particularly serotype O157:H5, have also been linked with an ulcerative colitis-like illness [9]. This study was originally designed to investigate the role of VTEC in community-acquired

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gastroenteritis and IBD, but we also took the opportunity to analyse the specimens for EAggEC and DAEC.

MATERIALS AND METHODS

In-patients from the Infectious Diseases (ID) Department or out-patients from the Gastroenterology Department were investigated. Stool specimens were obtained from consecutive patients who had not received antibiotics during the illness and fell into one of the following diagnostic categories: acute gastroenteritis presenting to the ID unit (with or without identified non-*E. coli* pathogens), IBD colitis at first presentation, in relapse or remission. A further eight patients with prolonged diarrhoea (> 2 weeks) after foreign travel were subsequently assessed as a separate group. Twenty-three acutely ill patients admitted to the ID unit who did not have diarrhoea, of whom 11 had recently been abroad, provided control specimens. IBD was defined as acute or chronic colitis with rectal histology and X-ray evidence of ulcerative colitis or Crohn's disease.

Stool was plated directly onto MacConkey agar, incubated overnight and the resulting colonies replicated onto nylon membranes. Bacteria were lysed, the DNA released, denatured and then bound to the membrane. The membranes were tested in DNA hybridization experiments using probes to detect VTEC, EAggEC and DAEC. Details of the probes and the conditions for preparation of membranes, hybridization and washings have been reported previously [10, 11]. Probe-positive organisms were identified biochemically and serotyped and also assessed for HEp-2 tissue culture adherence [3, 12].

Sera taken from the patients at the same time as the stool samples were assayed for antibodies against the lipopolysaccharide (LPS) antigens of *E. coli* O 157 and O 2 as previously described [13].

RESULTS

The diagnostic categories of the patients providing the 181 specimens, the *E. coli* sub-types identified and countries recently visited are displayed in Table 1. VTEC were not detected. The only 1 of 94 serum samples assayed for anti-O 157 LPS which was positive was from a patient with salmonella gastroenteritis and no clinical evidence of VTEC infection. Only 82 serum samples were tested for anti-O 2 LPS; all were negative.

All patients were adults except one control from Vietnam with EAggEC. Six of the eight EAggEC positive patients with diarrhoea, including one patient with IBD in relapse, had recently been abroad. Eight patients within the gastroenteritis (no pathogen isolated) group had diarrhoea for 14 or more days, of whom three were EAggEC probe-positive travellers (Table 1). Three EAggEC probe-positive patients, one each from the IBD relapse, control and acute gastroenteritis groups, had not recently travelled abroad, although the two EAggEC probe-positive control and gastroenteritis patients had arrived from Pakistan and Turkey respectively more than 6 months previously. The pathogens isolated in addition to probe-positive DAEC in the gastroenteritis (pathogen identified) group were *Salmonella* sp. (2), *Campylobacter* sp. (2) and *Shigella* sp. (1).

Table 1. E. coli *serotypes by patient's diagnostic category*

	EAggEC probe-positive isolates		DAEC probe-positive isolates	
Diagnosis	Serotype	Country where acquired*	Serotype	Country where acquired
A. Patients with diarrhoea				
Acute IBD (10)†	—		—	
IBD relapse (31)	O 78.H10		—	
	O 102.H27	SE Asia		
Gastroenteritis: (pathogen, identified) (40)	—		O 75.H- (3)	Malaysia Spain SE Asia
			O 5.H4	S. America
			O 15.H-	Indonesia
Gastroenteritis: (no pathogen isolated) (46)				
Diarrhoea	O ?.H1		O 21.H5	Cyprus
< 14 days (38)	O ?.H-	Kenya	O 75.H5	Algeria
Diarrhoea	O 33.H16	Russia		
> 14 days (8)	O 102.H27 (2)	Jamaica (2)		
Post-tropical chronic diarrhoea (8)	O 75.H2	Brazil	—	
B. Patients without diarrhoea				
Acute medical admissions (23)	O ?.H10 (2)	India	—	
	O 59.H-	Nigeria		
	O 151.H11	Vietnam		
IBD remission (23)	—		O ?.H- (1)	

* Infection UK-acquired unless otherwise indicated.

† Numbers in parentheses indicate the patients within each category if more than one.

Table 2. *Travel history of patients with gastroenteritis in relation to EAggEC and DAEC probe-positivity and detection of other bowel pathogens*

	Travel	No travel
No other pathogen identified	EAggEC +ve (4)	EAggEC +ve (1)
	DAEC +ve (2)	DAEC +ve (0)
	EAggEC/DAEC −ve (4)	EAggEC/DAEC −ve (35)
Alternative pathogen isolated	EAggEC +ve (0)	EAggEC +ve (0)
	DAEC +ve (5)	DAEC +ve (0)
	EAggEC/DAEC −ve (5)	EAggEC/DAEC −ve (30)

A precise travel history was obtained in all patients within the gastroenteritis group. In these 20 travellers, 6 of 10 (60%) with no other identified pathogen, and 5 of 10 (50%) of those in whom a non-*E. coli* pathogen was also identified, had EAggEC or DAEC in the stool (Table 2). One of 8 patients with prolonged post-

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tropical diarrhoea, who were assessed as a specific additional group, was also EAggEC probe-positive.

All DAEC probe-positive strains also showed diffuse adherence on HEp-2 tissue culture assay. Of 11 EAggEC probe-positive organisms tested, 5 displayed aggregative adherence, 6 did not adhere to HEp 2-cells. Three of the non-adherent strains were from controls and the other 3 were from patients with gastroenteritis. Two of the latter 3 patients with gastroenteritis were husband and wife.

DISCUSSION

VTEC were not isolated in patients with IBD included in this study in contrast to the previously reported evidence suggesting that these organisms may have an aetiological role [9]. The failure to detect VTEC in other patient groups supports published data that they cause under 2% of gastroenteritis in the UK [14]. It is possible that some cases could have been missed, as the rate of detection of VTEC in stools falls dramatically after the first few days of infection [15], and the VT property in *E. coli* O 2.H5 in particular can be unstable in culture [16]. However the absence of detectable antibodies to O 157 LPS or O 2 LPS in all but one of the serum samples from these patients is further evidence that VTEC were not present.

Eleven of the 12 probe-positive EAggEC were identified in 9 patients who had recently been abroad and 2 patients who had foreign connections. Eight had diarrhoea of whom 6 had recently spent a brief period abroad. Four of the 6 EAggEC positive travellers had prolonged diarrhoea, a symptom common to a high proportion of previously described patients with this infection [9]. The four without diarrhoea were immigrants from the tropics, a group that is frequently recognized to carry a variety of enteric pathogens asymptomatically [17]. Further studies are obviously required to clarify the possible link between these organisms and diarrhoeal disease, including IBD, in travellers.

There was poor correlation between EAggEC probe-positivity and tissue culture adherence, despite the 89% sensitivity and 99% specificity claimed for the probe [11]. However, the probe may be detecting a sub-group of *E. coli* for which aggregative adherence is not a uniform characteristic. The high rate of foreign travel, diarrhoea and prolonged illness amongst the probe-positive patients suggests that the probe was indeed detecting a true pathogen.

There was also an association with recent foreign travel in the eight DAEC positive patients. Some evidence from children living in the tropics suggests that DAEC are true pathogens causing acute watery diarrhoea [4], although other surveys have not confirmed this and in one challenge study DAEC failed to cause diarrhoea in human volunteers [3, 18]. Our data are equivocal on this point. All seven DAEC positive travellers had diarrhoea, but alternative bowel pathogens were also identified in five of these patients. This does not exclude a causal link between DAEC and travellers' diarrhoea, as such patients frequently acquire multiple pathogens [17].

It seems unlikely that VTEC are a major cause of IBD or a frequent cause of sporadic gastroenteritis in our patients. These results do, however, suggest a possible link between EAggEC, DAEC and travellers' diarrhoea. The possible

association between EAggEC and prolonged diarrhoea in returned travellers was strengthened by our findings. Further investigations in larger patient groups would clarify these points.

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